OECD GUIDELINES FOR TESTING OF CHEMICALS

TEST GUIDELINE 204

Test No. 204: Fish, Prolonged Toxicity Test: 14-Day Study

Information: Following the OECD Council decision, the Test Guideline 204 'Fish, Prolonged Toxicity Test: 14-Day Study' was deleted on 2nd April 2014.

Those who would like to obtain it should contact ehscont@oecd.org, or upload the file from the Internet page:

http://www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm#replaced_deleted_TGs .

"Fish, Prolonged Toxicity Test:
14-day Study"

2. METHOD

A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

This Test Guideline presents guidance for measurement of lethal and other observed effects in fish exposed to test substances. It may be used in place of Test Guideline 203 (Fish, Acute Toxicity Test) if a longer observation period is considered useful and appropriate and the reporting of additional information deemed necessary.

- Definitions

Semi-static test is a test without flow, but with occasional batchwise renewal of the test solution after prolonged periods (e.g. every 24 hours).

Flow-through test in this Test Guideline is a test in which water is renewed continuously in the test chambers, the test substance being transported with the water used to renew the test medium.

Threshold level of lethal effect is the lowest concentration of the test substance in the test solution at which the substance has a lethal effect.

Threshold level of observed effects is the lowest concentration of the test substance in the test solution at which the substance is observed to have an effect other than a lethal one on a significant number of test fish.

NOEC (no observed effect concentration) is the highest tested concentration of a test substance at which no statistically significant lethal or other effect is observed.

- Reference substances

No reference substances are recommended for this test. However, if a reference substance has been tested, the results should be given.

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• Principle of the test method

Threshold levels of lethal and other observed effects and NOEC are determined at intervals during the test period, which is at least fourteen days. If necessary, the test period should be extended beyond 14 days by one or two weeks.

• Conditions for the validity of the test

- The mortality in the controls should not exceed 10 per cent at the end of the test.
- The dissolved oxygen concentration should be at least 60 per cent of the air saturation value throughout the test.
- In semi-static procedures, aeration can be used, provided it does not lead to a significant loss of test substance.
- There should be evidence that the concentration of the substance being tested has been satisfactorily maintained (it should be at least 80 per cent of the nominal concentration) over the test period. The results should be based on measured concentrations if the deviation from the nominal concentration is greater than 20 per cent.

B. DESCRIPTION OF THE TEST PROCEDURE

• Preparations

Equipment

Normal laboratory equipment and especially the following is necessary:

- Equipment for determination of temperature, pH, oxygen concentration and hardness of water
- Adequate apparatus for temperature control
- Test tanks made of chemically inert material and of a suitable capacity

Solutions of the test substance

Stock solutions of the appropriate concentrations are prepared by dissolving the appropriate amount of the test substance in the required volume of dilution water.

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Stock solutions of test substances of low water solubility may be prepared by mechanical dispersion or, if necessary, by use of vehicles, such as organic solvents, emulsifiers or dispersants of low toxicity to fish. The concentration of organic solvents, emulsifiers or dispersants should preferably not exceed 100 mg/l in the test solution.

Test solutions of chosen concentrations are prepared by dilution of the stock solution.

The test should be carried out without adjustment of pH. If there is evidence of marked change in the pH of the tank water after addition of the test substance, it is advised that the test be repeated, adjusting the pH of the stock solution to that of the tank water before addition of the test substance. This pH adjustment should be made in such a way that the stock solution concentration is not changed to any significant extent and that no chemical reaction or physical precipitation of the test substance is caused. HCl or NaOH are preferred.

- Experimental animals

Selection of species

One or more species may be used, the choice being at the discretion of the testing laboratory. However, it is recommended that the species used for this test be selected from those recommended for the Acute Toxicity Test (Test Guideline 203). The species used should be selected on the basis of such important practical criteria as, for example, their ready availability throughout the year, their ease of maintenance, their convenience for testing and any relevant economic, biological or ecological factors. The fish should be in good health and free from any apparent malformation.

The fish mentioned in Test Guideline 203 are easy to rear and/or widely available throughout the year. They can be bred and cultivated either in fish farms or in the laboratory, under disease and parasite-controlled conditions, so that the test animal will be healthy and of known parentage. These fish are available in many parts of the world.

If other species fulfilling the above criteria are used, the test method should be adapted in such a way as to provide suitable test conditions.

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Holding

Acclimatisation:	At least 12 to 15 days. All fish must be exposed to water of the quality to be used in the test for at least seven days before they are used. Any disturbances that may change the behaviour of the fish should be avoided.
Water:	Drinking water supply (dechlorinated if necessary), good quality natural water or reconstituted water (see Test Guideline 203). Waters with a total hardness of between 50 and 250 mg of $CaCO_3$ per litre, and with a pH 6.0 to 8.5 are preferable. The reagents used for the preparation of the dilution water should be of analytical grade and the deionised or distilled water should be of conductivity equal to or less than 10 μScm^{-1}.
Light:	12 to 16 hours photoperiod daily.
Temperature:	Appropriate to the species (see Test Guideline 203).
Oxygen concentration:	At least 80 per cent of air saturation value.
Prophylactic treatment:	Prophylactic treatments should be avoided but reported when used.
Feeding:	Once daily
Mortality:	Following a 48-hours settling-in period, mortalities are recorded and the following criteria applied: – greater than 10 per cent of population in seven days: rejection of entire batch – between 5 and 10 per cent of population: acclimatisation continued for seven additional days – less than 5 per cent of population acceptance of batch

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- Performance of the test

If a vehicle is used in the preparation of the stock solution of the test substance, it is necessary to run, in addition to the control group, a control group of fish exposed to the highest concentration of the vehicle used in the test.

In the flow-through test, the concentration of the substance in the test solution may be determined at the beginning of the test; in the semi-static test at the beginning, immediately prior to the first renewal of the test solution and at the termination of the test. Appropriate procedures other than analysis for giving evidence that adequate concentrations of the test substance have been maintained can also be used.

Conditions of exposure

Duration:	Normally 14 days, but can be extended by one or two weeks.
Tanks:	Of suitable capacity in relation to the recommended loading.
Loading:	For semi-static tests maximum loading of 1.0 g fish/litre is recommended; for flow-through systems higher loading can be acceptable.
Number of animals:	At least 10 for each concentration and control.
Test concentrations:	The test concentrations chosen must permit the determination both of the threshold levels for the lethal and other observable effects and of the NOEC value. Concentrations of the substance in excess of 100 mg/l need not be tested if a threshold level has not been reached up to this concentration.
Water:	Drinking water supply (dechlorinated if necessary), good quality natural water or reconstituted water (see Test Guideline 203). Waters with a total hardness of between 50 and 250 mg of $CaCO_3$ per litre, and with a pH 6.0 to 8.5 are preferable.

The reagents used for the preparation of the dilution water should be of analytical grade and the deionised or distilled water should be of conductivity equal to or less than 10 μScm^{-1}.

Light: 12 to 16 hours photoperiod daily.

Temperature: Appropriate to the species (see Test Guideline 203) constant within ± 2°C.

Oxygen concentration: Not less than 60 per cent of the maximum air saturation value throughout the test.

Feeding: Either several times daily (the quantity of feed administered should not exceed the amount ingested immediately by the fish) or daily (the quantity of food being kept constant - e.g. 2 per cent dry weight related to the initial fish weight).

Cleaning: Inside surfaces of the test tank in the flow-through test must be cleaned if necessary and the remaining excrement removed, at least twice weekly; in the semi-static test the test tank is replaced by a clean one each time the water is changed.

Observations

Observed effects are defined as follows:

Lethal effects: a fish is presumed to be dead if no respiratory movement and no reaction to a slight mechanical stimulus can be detected.

Effects other than lethal effects: these include all effects observed on the appearance, size and behaviour of the fish that make them clearly distinguishable from the control animals, e.g. different swimming behaviour, different reaction to external stimuli, changes in appearance of the fish, reduction or cessation of food intake, changes in length or body weight.

The fish are inspected at least once a day for mortality. Dead fish are removed when observed and mortalities are recorded.

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It is desirable that daily records be kept of all observed effects, but a minimum of three observation sessions per week must be conducted.

Measurements of pH, dissolved oxygen and temperature must be carried out at least twice a week.

Representative samples of the test population should be weighed and measured before the test starts. All survivors should be weighed and measured at the termination of the test. Fish should not be weighed or measured during the test as unnecessary handling may lead to damage and/or mortality.

3. DATA AND REPORTING

- Interpretation of results

If it is observed that the stability or homogeneity of the test solutions cannot be maintained, care should be taken in the interpretation of the results and note made that these may not be reproducible.

- Test report

The test report should include the following information:

Test substance: chemical identification data.

Test organisms: scientific name, strain, size, supplier, any pretreatment, etc.

Test conditions:

- test procedure used (e.g. semi-static or flow-through, aeration, fish loading, etc.)
- water quality characteristics (treatment, including declorination, dissolved oxygen concentration, pH, hardness, temperature, any other information available)
- dissolved oxygen concentration, pH values, temperature and total hardness of the test solutions at each of the recommended observation times

- methods of preparation of stock and test solutions
- concentrations used
- information on the maintenance of the concentration of the test substance in the test solutions
- number of fish at each test concentration

Values from the fish acute toxicity test

Results:

- observed effects at each concentration for each observation time in tabular form
- concentrations that produce lethal or other effects can be presented graphically with respect to time
- Threshold level of lethal effect
- Threshold level of observed effects
- NOEC
- cumulative mortality at each concentration and for each recommended observation time if possible
- mortality in the controls
- behavioural observation of the fish
- incidents in the course of the test which might have influenced the results
- any deviation from the Test Guideline

4. LITERATURE

1. D.M.M. Adema, in *Degradability, Ecotoxicity and Bioaccumulation*, Chapter 5, Government Publishing Office, The Hague (1980).

2. R. Bathe, *Arch. Toxicol. Suppl.* 2, 417-423 (1979).